aligned with the MT axis, indicating the existence of a shorter time-scale transition period on the MT before free diffusion begins. We also correlated the changes in early endosome motion with the position of other organelles and the cytoskeleton. Pauses in directed movement spatially correlate with regions of dense MTs as well as other early endosomes and the endoplasmic reticulum, suggesting that early endosomes interact with these cellular features during their transport.
Supported by NIH P01GM087253.

**3348-Pos Board B503**
**Time-Lapse Super-Resolution Imaging of Apical Membrane Protein Domains in Live Filamentous Fungi**
**Yuji Ishitsuka**[1], Yiming Li[1], Reinhard Fischer[1], Norio Takeshita[1], G. Ulrich Nienhaus[1,2].
[1]Karlsruhe Institute of Technology, Karlsruhe, Germany, [2]University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The core mechanism of the asymmetric and the polarized cell growth is a conserved process among many organisms. This process involves complex concerted interplay of dynamic rearrangements of cytoskeletons, the mobilization of proteins from intracellular pools, active transport of vesicles to fusion sites and the accumulation of ''cell end marker'' proteins. While many of the key components for the polarized growth are known, the detailed mechanism of these complex processes is still unclear. TeaR is one of the cell end marker protein that plays a crucial role in the initiation and the maintenance of straight-growth in a filamentous fungi, *Aspergillus nidulans*. While many end marker proteins show a single accumulation of the domain near the end of the cell, widefield fluorescence microscopy data shows a distribution of multiple TeaR domains. Our current colocalization studies strongly suggest that majority of TeaR domains consist of accumulated secretory vesicles that are docked near the plasma membrane. This suggests that these domains mark the exocytosis sites of the hypha. In order to elucidate the detailed architecture of TeaR domains near the hypha tip, we have performed a super-resolution microscopy imaging, photoactivated localization microscopy (PALM) in live filamentous fungi. PALM imaging of TeaR fused with a photoconvertible fluorescent protein, mEosFP*thermo*, shows a cluster size distribution centered around 140 nm. Time-lapse PALM imaging further reveals that these clusters in growing cells are highly dynamic. Processes resemble vesicle trafficking along the cytoskeleton, docking, accumulation of vesicles, dispersion of proteins along the membrane and the growth of the membrane has been observed. We present the affect of key deletion mutants that influence the directionality of the hypha growth on these dynamics.

**3349-Pos Board B504**
**Magnetic Manipulation of Axonal Transport in Live Neurons**
**Praveen D. Chowdary**, Chong Xie, Yasuko Osakada, Dung L. Che, Bianxiao Cui.
Stanford University, Stanford, CA, USA.
Retrograde neurotrophic signals, from the axon terminal to the cell body, are essential for the survival and function of neurons. Axonal microtubules serve as polarized tracks for molecular motor proteins driving the signaling endosomes from the axon terminal to the cell body. The robustness of this long-distance transport and the direction specificity can be attributed to the cooperative mechanics of multiple motors and/or specific coordinators in vivo. Noninvasive external force control of axonal endosomes in live neurons is a challenging prospect, which can unravel the transport machinery and the direction regulation mechanisms in vivo. Here, we present an integrated methodology based on microfluidic neuron culture, high-gradient magnetic trapping and pseudo-TIRF imaging that permits external control of axonal endosome transport in live neurons via magnetic forces. We fabricated a novel microfluidic device for neuron culture by patterned electrodeposition of soft micromagnets on glass coverslips. In the presence of an external magnetizing field, the soft micromagnetic pattern gives rise to local zones of high magnetic gradients. By culturing neurons in this device, with axons aligned along these high gradient zones, we can exert pN forces on axonal endosomes carrying magnetic nanoparticles (<100 nm). The magnetic forces can be designed to either assist/oppose the molecular motor forces driving the axonal endosomes. We have successfully compartmentalized DRG neurons in prototype magnetic devices. Further, high-resolution tracking of axonal endosomes under external load and stochastic modeling reveal that A) motors of both polarity are involved even in the apparent unidirectional transport of axonal endosomes and B) mechanical force balance is a critical component in determining the endosome directionality.

**3350-Pos Board B505**
**Kinesins in Caenorhabditis Elegans Chemosensory Cilia Relay to Drive Intracellular Transport**
Bram Prevo[1], **Pierre Mangeol**[1], Felix Oswald[1], John M. Scholey[2], Erwin J.G. Peterman[1].
[1]VU university Amsterdam, Amsterdam, Netherlands, [2]UC Davis, Davis, CA, USA.
Cilia are organelles emanating from the surface of nearly every cell in the body playing crucial roles in cell signaling and motility. For cilium development and maintenance, intraflagellar transport (IFT) along the ciliary axoneme is essential. In Caenorhabditis elegans chemosensory cilia, IFT is driven by two kinesin-2's - kinesin-II and OSM-3 - carrying cargo toward the tip of the cilium and a cytoplasmic dynein - dynein 1b - driving the transport in the opposite direction. How these motor proteins cooperate and how their action is regulated is largely unknown. Here we apply high-sensitivity, quantitative wide-field fluorescence microscopy, which allows visualization of fluorescent motor proteins at endogenous expression levels. To this end, we have generated transgenic worms using Mos1-mediated single-copy integration of transgenes encoding fluorescently-labeled IFT-kinesins. We show that kinesins relay on the cilium structure to drive IFT: kinesin- II is the key player in the initial stage, while OSM-3 takes over further on. At the base of the tip, kinesin-II combines with tens of other kinesin-II's to form trains of motors. On the so-called middle segment of the cilium, kinesin-II motors progressively detach from the microtubules and are almost instantly transported back. At the same time, OSM-3 motors increasingly take over and transport cargo further, to the cilium tip. These findings shed new light on cooperativity of motor proteins driving intracellular transport in vivo.

**3351-Pos Board B506**
**Huntington-Associated Phosphorylation of Kinesin-1 Enhances Autoinhibition in a Phosphomimic**
**David D. Hackney**, Young Yeo, Calvin T. Lin.
Carnegie Mellon University, Pittsburgh, PA, USA.
One of the consequences of the triplet expansion in Huntington's disease is inhibition of fast axonal transport (FAT). Phosphorylation of Ser176 in human kinesin-1 by JNK3 has been implicated in this inhibition (Morfini, et al., Nature Neruo. 12, 866 (2009)). To investigate the molecular basis for the inhibition of FAT, we have generated the S182E phosphomimic of the homologous residue in *Drosophila* kinesin-1. When introduced into short dimer of motor domains, the S182E mutation produces a 30% decrease in the maximum rate of microtubule-stimulated ATPase rate in solution and a similar reduction in the sliding rate of axonemes in a multimotor sliding assay. This only modest decrease suggests that direct inhibition of motility is not likely to be the principal cause of the pronounced inhibition of FAT. However, free kinesin is known to be autoinhibited through the binding of a tail domain to a dimer of motor domains (heads) and the Ser182 phosphorylation site is near the tail binding site on the heads (Kaan, et al., Science 333, 883 (2011)) where it could influence autoinhibition. One possibility is that the increased negative charge on the heads due to phosphorylation of Ser182 could produce a stronger interaction with the positively charge tail domain that would strengthen autoinhibition and inhibit FAT. To test the effect of the phosphomimic on autoinhibition, the binding of a monomeric tail domain to a dimer of motor domains was determined using a FRET assay. In 100 mM KCl, monomeric tail domains bind to mutant S182E heads three-fold more tightly than to wild type heads. This suggest that inhibition of FAT may principally be due to enhanced tail binding and accompanying autoinhibition of kinesin-1 following phosphorylation.
Supported by NIH Grant NS058848.

**3352-Pos Board B507**
**Biomimetic Cilia as a Model Ependymal Cilia System**
**Michael Berg**.
Elon University, elon, NC, USA.
Cilia are ubiquitous throughout the human body and serve a variety of functions. Human lung cilia in particular have been widely studied due to the prevalence of ciliary diseases such as cystic fibrosis. Less-well-studied are ependymal cilia, which are responsible for transporting cerebrospinal fluid throughout the ventricular system; however, their response to increased viscous loading during infection may be critical in understanding the pathology and treatment of meningitis and other inflammatory diseases.
While ependymal cilia and human lung cilia are morphologically homologous, it has been shown in ex vivo studies that they respond very differently to increased viscous loading: lung cilia maintain a constant beat frequency but show decreased beat amplitude, while ependymal cilia maintain amplitude